BIOLOGY OF LACEWING BUG *Leptopharsa gibbicarina* AND SELECTION OF FUNGAL ENTOMOPATHOGENS TO CONTROL ITS POPULATIONS IN OIL PALM PLANTATIONS IN COLOMBIA

Alex Enrique Bustillo Pardey[1], Carlos Enrique Barrios Trilleras[2], Maira Selene Cuchimba Triana[3], Kely Lorena Ocampo Rodas[3]

Pest and Diseases Research Program. Colombian Oil Palm Research Centre, Cenipalma, Calle 20ª No. 43ª – 50, Bogotá, Colombia.

1. abustillo@cenipalma.org; 2. cbarrios@cenipalma.org; 3. Student research assistant.

ABSTRACT: Oil palm plantations in Colombia are affected by several insect pests, but those who are associated to plant diseases are of capital importance. This is the case of the lacewing *Leptopharsa gibbicarina* which when feed on the foliage of palms, open the entrance for several fungi of the genera: *Pestalotiopsis*, *Colletotrichum*, *Gloeosporium* and *Helminthosporium*, which cause a wilting on the foliage called "Pestalotiopsis". Studies on the life cycle (28 °C, RH 85%) of *L. gibbicarina* have shown that egg hatching takes 16 ± 2 days, nymphs lasted 18.9 ± 3.3 days and adult stage lasted 37.2 ± 5.9 days. Females oviposited eggs individually on underside of leaf and attached to the parenchyma. These studies were basic to establish a colony of this insect to test entomopathogenic fungi. Initially a pathogenicity test was carried out to select several fungi isolates: *Isaria fumosorosea* (CPIf1001), *Purpureocillium lilacinus* (CPPl0601) and *Beauveria bassiana* (CPBb0404). These tests showed that all of them were able to produce disease in the *L. gibbicarina* population tested. Then a virulence test was performed using young oil palms, (27.8 ± 3.2°C, RH 84,7 ± 13,2%, and 9.7 mm of rain), which were previously infested with adults of *L. gibbicarina* and sprayed with these fungi using a dosage of 1×10^{13} conidia/ha. There were significant differences among treatments; greatest mortality (100%) was caused by *P. lilacinus*, followed by *B. bassiana* (92.9%), which were different from *I. fumosorosea* (74.4%). A further experiment to test three dosages (5×10^{12}, 1×10^{13}, 1.5×10^{13}, conidia/ha), was conducted and results showed that there were no statistical differences among dosages and fungi isolates. Mortality for isolates *P. lilacinus* and *B. bassiana* were higher than 88%, and are considered for further research to test them under commercial plantations using a dosage of 1×10^{13} conidia/ha.

Key words: *Elaeis guineensis*, Entomopathogenic fungi, Biological control.

1. INTRODUCTION

Leptopharsa gibbicarina Froeschner (Hemiptera: Tingidae) is an insect that attacks oil palm plantations in Colombia and it is distributed in the Central and North Oil Palm Zones of Colombia (Jiménez, 1980). *L. gibbicarina* causes a direct damage by sucking the sap of leaflets which gives rise to chlorotic lesions. These areas are invaded by fungi like *Pestalotiopsis palmarum* Cooke & Poor. Initially symptoms are on the underside of leaf, forming small circular brown pecks which finally are necrosed (Zenner de Polanía y Posada, 1992; Genty *et al*. 1978; Labarca *et al*. 2006). The complex *Leptopharsa* – *Pestalotiopsis*, can cause reduction in the productivity of oil palm, which can reach up to 36% (Jiménez y Reyes, 1977; Labarca *et al*. 2006).

Several natural enemies of *L. gibbicarina*, have been registered; among them are ant predators such as *Crematogaster* sp., *Camponotus* sp. and *Ectatomma* sp. (Hymenoptera: Formicidae) (Medina y Tovar, 1997; Aldana *et al*. 1995), also several species of *Chrysopa* sp. (Neuroptera: Chrysopidae), and species of Coccinellidae and Carabidae (Guzman *et al*. 1997; Medina and Tovar, 1997; Aldana *et al*. 1995). Entomopathogenic fungi like *Beauveria bassiana*, *Isaria* sp. (Valencia and Benítez, 2005) and *Sporotrix insectorum* (Ordoñez and

Genty 1989) are recorded. However, the most common control measures are insecticides which are injected to the trunk, or by radicular absorption. This practice results in increased costs, development of resistance and negative environmental effects. Therefore, searching for alternative management practices are needed (Méndez, 2000). There is a lack of knowledge on the biology and biological control of *L. gibbicarina* under oil palm plantation conditions, so the objective of this research was to study and understand its bionomics and determine the efficacy of entomopathogenic fungi to control their populations.

2. MATERIALS AND METHODS

2.1. Location.

This research was conducted between February 2012 and December 2013, in the oil palm plantation "Palmeras de la Costa S.A.", located at the municipality of El Copey (Cesar) (10° 9' Latitude north and 73° 28' Longitude west) and at an altitude of 180 m.a.s.l.

2.2. Rearing L. gibbicarina.

To establish a rearing system for *L. gibbicarina*, 27 oil palms 18 month old were placed under a shaded area (16 m long by 7 m wide) in an oil palm plantation (Fig.1). Sleeve cages were placed on oil palm leaves and about 80 nymphs of *L. gibbicarina* were introduced in each cage, so they can reach the adult stage and lay eggs. To study the life cycle, a total of 200 eggs were selected and development of nymphs to adult stage was recorded. Number of adults, oviposition and egg viability were also recorded. Data were analyzed through descriptive statistics.

2.3. Life cycle.

Once the *L. gibbicarina* rearing was established, the life cycle was initiated by following a cohort of 200 eggs and recording the time spent in the development of the insect stages and adult longevity. Instar change was identified by daily observations of shed exuvia by the insect. Data were analyzed through descriptive statistics.

2.4 Population parameters.

To determine the population parameters of *L. gibbicarina*, 40 leaflets of oil palm were concealed in a translucent acetate tube (42 cm length, 5 cm diameter), with extremes of the tube covered with a silk cloth. Inside these tubes, eight adults of *L. gibbicarina* were introduced and placed on the leaflets of the palm (Fig. 2). Adults were left in these tubes for oviposition during 24 h, and insect development was followed on these cohorts, recording duration, survival of each stage, eggs deposited and viability. This information was useful to estimate the population parameters of *L. gibbicarina* (Bellows *et al*. 1992, Arce *et al*. 2006). Environmental conditions (Temperature, relative humidity and precipitation) were recorded during the study.

Figure 1. Oil palm plants concealed in a shaded area in a plantation. Observe the sleeve cages to follow up populations of *Leptopharsa gibbicarina* (Photo C. Barrios, Cenipalma),

Figure 2. Oil palm leaflets concealed in an acetate tube to follow development of *Leptopharsa gibbicarina* (Photo K. Ocampo, Cenipalma).

2.5. Pathogenicity experiment.

A test of pathogenicity was made using several entomopathogenic fungi from the "Insect Entomopathogenic Laboratory Collection of Cenipalma", previously isolated from infected insects in oil palm plantations in Colombia. Three fungi strains were chosen: *Isaria fumosorosea* (CPIf1001), *Purpureocillium lilacinus* (CPPl0601) and *Beauveria bassiana* (CPBb0404) (Table 1). A Petri dish laboratory bioassay was prepared pouring 10 ml of water agar media (2%) and covering it with disinfected pieces of oil palm leaves, on which individual lacewing adults coming from the rearing unit, were placed. Fungi preparations were set at a concentration of $1x10^7$ conidia/ml and 0.2 ml was applied with a manual sprayer. Insects were observed daily and mortality registered. The experiment was organized under a completed randomized design with seven repetitions, being the Petri dish the observational unit and 10 of these units conformed the experimental unit.

Table 1. Fungi species tested for pathogenicity to *Leptopharsa gibbicarina.*

Treatment	Code	Original host	Locality
Isaria fumosorosea	CPIf1001	Adult of *L. gibbicarina*	Fundación, (Magdalena)
Purpureocillium lilacinus	CPPl0601	Unknown	Meseta San Rafael (Santander)
Beauveria bassiana	CPBb0404	Larva of *Stenoma cecropia*	San Andrés de Tumaco (Nariño)

2.6. Virulence experiment.

The virulence experiment of selected pathogenic fungi to *L. gibbicarina* was conducted under shade oil palms in a plantation, to determine the efficacy to control adults of this pest. The concentration of fungi strains were adjusted to $2.3x10^8$ conidia/ml to apply an equivalent dosage of $1x10^{13}$ conidia/ha. Treatment application was made with a manual sprayer. The experiment was organized under a completed randomized design with seven repetitions and the experimental units were 10 units of observations, conformed by an acetate tube with an oil palm leaflet and 8 adults of *L. gibbicarina* taken from the rearing unit. Mortality was recorded daily after the fourth day of treatment application

2.7. Dosage experiment.

To determine dosage of previously selected entomopathogenic fungi to control adults of *L. gibbicarina,* under shade oil palms in a plantation, an experiment was organized under a complete randomized block design with five repetitions Three dosages were evaluated ($5x10^{12}$, $1.0x10^{13}$, $1.5x0^{13}$ conidia/ha). The experimental unit was one sleeve cage/palm leaf with 50 adults of *L. gibbicarina* taken from the rearing unit. Treatment applications were made with a sprayer with a capacity of 1.5 liters, and a surfactant was added (1 cc/l) to the fungi suspension. Daily observations were made after the second day of treatment application and adult mortality of *L. gibbicarina* was recorded.

3. RESULTS AND DISCUSSION.

3.1. Rearing L. gibbicarina.

Rearing was made under a shade field house in an oil palm plantation successfully and was maintained for ten generations with levels of about 3000 individuals per generation. This rearing system allowed experiments on insect life history and evaluations of entomopathogenic fungi for population control of *L. gibbicarina*. The environmental conditions during the study were 27.3 ± 5.7°C, and 84.5 ± 8.8% of RH, and an accumulative rain of 183 mm.

3.2. Life cycle.

Results on life cycle of *L. gibbicarina* are shown in table 2. The insect rearing process allowed the study of biology under shade palms in the field. Average total life cycle lasted 72.1 days (27°C, 85% RH). Sex ratio was 1:1. Female *L. gibbicarina* only oviposited isolated eggs on underside of the leaf, and attached to the leaf parenchyma. One female oviposited on average 39.7±2.5 eggs. Egg stage lasted 16 days, nymph stage took 18.9 days and underwent five instars; adult stage

lasted 37.2 days. This information is similar to the one registered to *Leptopharsa* by other authors, Genty *et al*. (1978) for *L. gibbicarina* and Cividane *et al*. (2004) for *L. heveae* pest of *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. In general differences are due to dissimilarities of hosts and environmental conditions in the studies.

Table 2. Life cycle of *Leptopharsa gibbicarina* under an oil palm plantation conditions (27±3.8°C, 85±15% HR).

***L. gibbicarina* stages**	**N***	**X** ± D.E. (Days)**	**Range (min–max.) (Days)**
Egg	200	16.0 ± 2.0	14.0 – 18.0
I Instar nymph	157	3.3 ±0.5	3.0 – 5.0
II Instar nymph	147	3.3 ± 0.5	3.0 – 4.0
III Instar nymph	142	3.3 ± 0.5	3.0 – 5.0
IV Instar nymph	128	4.2 ± 0.8	3.0 – 6.0
V Instar nymph	115	4.8 ± 1.0	3.0 – 7.0
Total nymph		18.9	-
Egg to Adult		34.9	-
Adults	99	37.2 ± 5.9	24.0 – 48.0
Total		72.1	

*N: number of individuals
**X: average duration in days

3.3. Population parameters.

Population parameters estimation of *L. gibbicarina* is presented in table 3. The rate of mortality (q_x) was the greatest in the egg stage ($q_x = 0.22$) followed by the V instar nymph ($q_x = 0.14$); while the lower rates of mortality were found in nymphal I ($q_x = 0.06$) and II ($q_x = 0.03$). Something similar was registered for *Gargaphia torresi* Costa Lima (Arce *et al*. 2006). The net reproductive rate (Ro) was 10, indicating that *L. gibbicarina* has a great reproductive rate, similar to other important pests reared under laboratory conditions (Romero and Cortina, 2007; Yang and Chi, 2006). The intrinsic rate of growth (Ro) of *L. gibbicarina* was 0.03 while the generation time was 33.2 days.

Table 3. Population parameters of *Leptopharsa gibbicarina* estimated under a shade condition in an oil palm plantation (27 ± 3.8°C, 85 ± 15 % HR).

Stage (X)	n_x	d_x	q_x	l_x	m_x	R_o
Egg	200	43	0.22	1.00	-	
Nymph I	157	10	0.06	0.79	-	
Nymph II	147	5	0.03	0.74	-	
Nymph III	142	14	0.10	0.71	-	
Nymph IV	128	13	0.10	0.64	-	
Nymph V	115	16	0.14	0.58	-	
Adult	99	99	1.00	0.50	20	10

X: age class; $\mathbf{n_x}$**:** numbers of individuals surviving at start of of age interval X; $\mathbf{d_x}$**:** number of individuals dying between ages X and X+1; $\mathbf{q_x}$**:** probability of dying between X and X+1; $\mathbf{l_x}$**:** proportion of surviving at age X; $\mathbf{m_x}$**:** average number of female progeny produced by every female at this age; **Ro** Intrinsic rate of development.

3.4. Pathogenicity experiment.

All fungi strains tested for pathogenicity *I. fumosorosea*, *P. lilacinus* and *B. bassiana* were pathogenic to *L. gibbicarina*. No statistical differences were found among treatments. Under the experiment conditions, all of them reached mortalities above 90%, 4 days after treatment. Mortality was observed the next day of fungi application and was increased at the third day (Table 4). This high mortality was due probably to a previous reactivation of all fungi strains, which were cultivated in media enriched by cadavers of *L. gibbicarina*. These findings have been documented in other studies as mentioned by Bustillo y Marin (2002) and González *et al*. (1993, 2001).

Table 4. Percent mortality of *Leptopharsa gibbicarina* caused by entomopathogenic fungi after several days of treatment application in a laboratory experiment to test pathogenicity.

Treatments	% Mortality (days after treatment)				
	1	2	3	4	5
Isaria fumosorosea	11.4	35.7	80.0	100.0	100.0
Purpureocillium lilacinus	7.1	24.3	78.6	97.1	97.1
Beauveria bassiana	10.0	30.0	70.0	97.1	97.1
Control	0.0	1.4	4.3	5.7	5.7

3.5. Virulence experiment. Results of this experiment are shown in figure 1. The environmental conditions during this experiment were 27.8±3.2°C, 84.7±13.2% RH and a precipitation of 9.7 mm. There were significant differences among treatments according to the Duncan test (P=0.05), greatest mortality (100%) to *L. gibbicarina* was caused by *P. lilacinus*, followed by *B. bassiana* (92.9%) which were different from *I. fumosorosea* (74.4%) (Fig. 3). However all strains were considered for a new experiment to determine the most effective dosage to control populations of the lacewing.

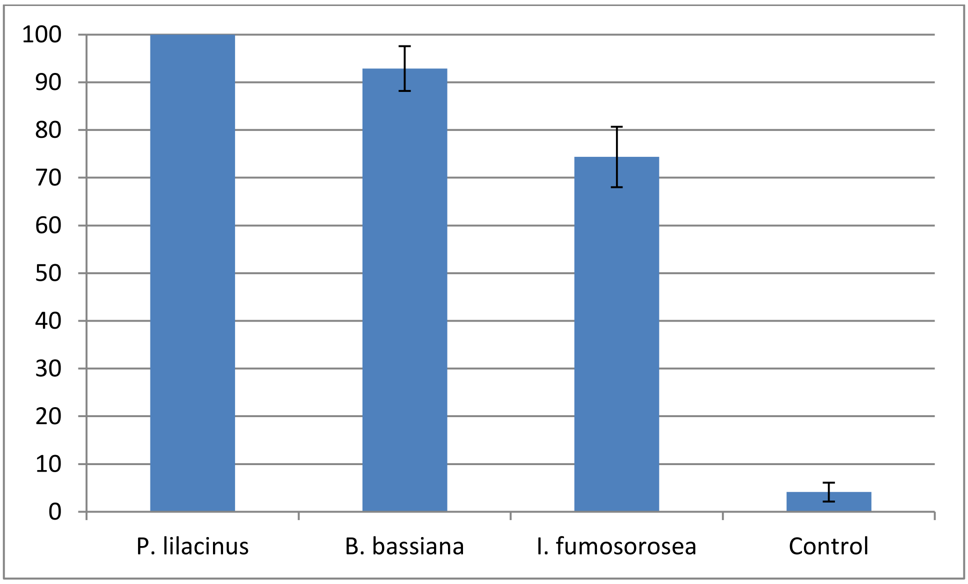

Figure 3. Virulence experiment. Percent mortality of *Leptopharsa gibbicarina* 10 days after application of entomopathogenic fungi at a dosage of $1x10^{13}$ conidia/ha. (27.8°C, HR 84.7% RH, precipitation 9.7 mm).

3.6. Dosage experiment. This experiment was conducted by spraying the selected strains of fungi *P. lilacinus, B. bassiana* and *I. fumosorosea* at three different dosages ($5x10^{12}$, $1x10^{13}$, $1.5x10^{13}$, conidia/ha) in a shaded area of an oil palm plantation. The environmental conditions were 27.3 ± 5.7°C, 84.5 ± 8.8% RH, and precipitation of 183 mm. Results are shown in table 5. There were no statistical differences among dosages and fungi isolates. Mortality for isolates *P. lilacinus* and *B. bassiana* were higher than 88%, and are considered for further research to test them under commercial plantations using a dosage of $1x10^{13}$ conidia/ha.

Table 5. Percent mortality of *L. gibbicarina* caused by entomopathogenic fungi applied at three different dosages 14 days after treatment applications.

TREATMENTS	**Dosage conidia/ha**		
	$1.5x10^{13}$	**$1.0x10^{13}$**	**$5.0x10^{12}$**
Isaria fumosorosea	88.8 a	94.0 a	82.5 a
Beauveria bassiana	94.7 a	93.1 a	92.0 a
Purpureocillium lilacinus	88.6 a	92.2 a	77.7 a
Control	8.0 b	8.0 b	8.0 b

Results of these experiments on the effect of entomopathogenic fungi against populations of *L. gibbicarina* allow confirming that the three species *I. fumosorosea, B. bassiana* and *P. lilacinus,* are pathogenic and have a greater virulence against *L. gibbicarina* populations with levels of control above 80%. But comparing the lethality of these strains through time, it was found that *P. lilacinus* and *B. bassiana* are more efficient causing a greater mortality in the first 3 days after treatment application. In order to continue this research under field conditions in commercial plantations these two species are considered.

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